



# Obstructive sleep-disordered breathing and plasma levels of vascular endothelial growth factor in children<sup>☆</sup>

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## KEYWORDS

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**Summary** Vascular endothelial growth factor (VEGF) may be one of the pathophysiologic links in the association between obstructive sleep apnea-hypopnea and cardiovascular disease. Morning serum VEGF levels are increased in children with obstructive sleep apnea. However, release of VEGF by platelets and leukocytes during blood clotting may affect its concentration in serum. In the present study, VEGF levels were measured in children with and without habitual snoring using plasma specimens. Evening and morning plasma VEGF concentrations were determined in: (i) 20 children with habitual snoring and apnea-hypopnea index (AHI)  $\geq 5$  episodes/h (median age 5; range 1.9–13 years); (ii) 55 children with snoring and AHI  $< 5$  episodes/h (median age 6; 2–13 years); and (iii) 25 controls without snoring (median age 6.5; 3–13 years). No differences were identified between the three study groups regarding evening [median 2.5 (range 2.5–174.5) versus 22.5 (2.5–159.4) versus 26.8 (2.5–108) pg/mL;  $P > 0.05$ ] and morning VEGF levels [median 7.7 (range 2.5–120.5) versus 25.1 (2.5–198.4) versus 48.4 (2.5–147.7) pg/mL;  $P > 0.05$ ]. AHI and % sleep time with oxygen saturation of hemoglobin less than 90% were not significant predictors of log-transformed morning VEGF concentrations ( $P > 0.05$ ). In summary, both evening and morning plasma VEGF levels were similar in children with obstructive sleep-disordered breathing of variable severity and in

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controls without snoring. VEGF may not play an important pathophysiologic role in all cases of obstructive sleep-disordered breathing in childhood.  
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## Introduction

Significant correlations between obstructive sleep-disordered breathing and cardiovascular disease have been reported in adults. Even mild increases in the apnea-hypopnea index (AHI) (1–10 episodes/h) have been associated with higher risk for coronary artery disease, congestive heart failure and stroke.<sup>1</sup> Among other variables, systemic hypertension,<sup>2</sup> chronic inflammation and metabolic disturbances,<sup>3,4</sup> endothelial dysfunction<sup>5</sup> and changes in myocardial structure and function<sup>5</sup> have been implicated in the interaction between cardiovascular disease and sleep apnea. Similar abnormalities have been described in habitually snoring children.<sup>6–10</sup>

Vascular endothelial growth factor (VEGF), a cytokine stimulating angiogenesis,<sup>11</sup> may contribute to the process of atherogenesis and development of cardiovascular disease.<sup>12–15</sup> Its morning concentrations are increased in adults with obstructive sleep apnea-hypopnea.<sup>16–18</sup> Treatment of sleep apnea with nasal continuous positive airway pressure or of the associated hypoxemia with administration of oxygen have been accompanied by a significant decrease in morning values of VEGF.<sup>18,19</sup>

To our knowledge, there is only one report on morning serum levels of VEGF in children with obstructive sleep-disordered breathing.<sup>20</sup> Higher levels of VEGF have been detected in children with sleep apnea (apnea index > 5 episodes/h) than in control subjects.<sup>20</sup> Nevertheless, platelets and leukocytes release VEGF during the process of blood clotting and for this reason measured concentrations of the factor are higher in serum samples than in plasma samples.<sup>20,21</sup> Furthermore, VEGF concentrations are not consistently increased in all adults with obstructive sleep-disordered breathing; in a recent study by Valipour and colleagues,<sup>22</sup> similar plasma VEGF levels were reported in adult participants with and without obstructive sleep apnea. The aim of the present investigation was to measure evening and morning plasma VEGF levels in children with obstructive sleep-disordered breathing of variable severity and in controls without snoring. We hypothesized that severity of sleep-disordered breathing is related to morning plasma VEGF concentration. To our knowledge, this is the first study assessing both evening and morning VEGF levels in children with sleep apnea.

## Patients and methods

### Study design

Consecutive children, who were referred to the Sleep Disorders Laboratory because of habitual snoring (more than three nights/week) present for at least 6 months, were recruited in the study. Healthy children without history of snoring that were admitted to the pediatric inpatient service for constipation or functional abdominal pain were also recruited as controls. Children were excluded from participation in the study if they had: (1) symptoms or signs of acute or chronic inflammation; (2) lung disease associated with hypoxemia; (3) cardiovascular disease; and (4) neuromuscular or genetic disorders.

The study protocol was approved by the Ethics Committee of the Larissa University Hospital. Informed consent was obtained from parents and child's assent from subjects older than 7 years. Since January 2003, more than 200 pediatric polysomnograms are performed in the Sleep Disorders Laboratory of the Larissa University Hospital annually. None of the subjects in the present investigation were included in other research projects that were carried out in our center.<sup>23,24</sup>

Parents were interviewed and information about symptoms of sleep-disordered breathing and about past medical history was collected. All participants underwent a physical examination. Polysomnograms were performed overnight in the Sleep Disorders Laboratory for all children with snoring but not for controls. The Alice-4 computerized system (Healthdyne, Marietta, GA, USA) was used.

Sleep stages and arousals were determined using standard criteria.<sup>25,26</sup> Obstructive apnea was defined as the presence of chest/abdominal wall motion in the absence of airflow for at least two breaths in duration.<sup>27</sup> Hypopnea was: (1) a reduction in the airflow signal amplitude of at least 50% compared to baseline; (2) in the presence of chest/abdominal wall motion; and (3) associated with oxygen desaturation of hemoglobin equal to or greater than 4% or with an arousal.<sup>27</sup> AHI, respiratory movement/arousal index, oxygen desaturation of hemoglobin index, oxygen saturation of hemoglobin nadir and % sleep time with oxygen saturation of hemoglobin < 90% were calculated as described previously.<sup>23,24</sup>

## VEGF measurements

Since platelets and leukocytes release VEGF during blood clotting,<sup>21</sup> measurements were carried out in plasma and not serum specimens. Evening and morning blood samples were collected into glass tubes containing EDTA, were immediately centrifuged, and the supernatant (platelet-free plasma) was aliquoted and frozen at  $-70^{\circ}\text{C}$  until assay.

A commercially available ELISA kit for the in vitro determination of human VEGF-165 and VEGF-121 was used (Human VEGF; BioSource Europe S.A., Nivelles, Belgium). The minimum VEGF concentration detected by this assay is 5 pg/mL. Intra-assay coefficients of variation are 5.5% at 87.4 pg/mL, 3.7% at 345 pg/mL and 4.9% at 938 pg/mL.

## Data analysis and statistics

Three groups of subjects were studied: (1) children with habitual snoring and  $\text{AHI} \geq 5$  episodes/h<sup>8</sup>; (2) children with habitual snoring and  $\text{AHI} < 5$  episodes/h; and (3) control subjects without snoring. There have been no previous studies in children with sleep-disordered breathing reporting VEGF concentrations in plasma specimens. Therefore, it is not possible to calculate the sample size that is required to detect a significant difference in VEGF levels between subjects with an  $\text{AHI} \geq 5$  episodes/h and subjects with an  $\text{AHI} < 5$  episodes/h.

Overnight change in VEGF levels was defined as: morning plasma VEGF concentration – evening plasma VEGF concentration. Values below the lowest detection limit of the method (5 pg/mL) were considered equal to 2.5 pg/mL. The Kruskal–Wallis test was applied to compare the three groups regarding continuous characteristics. Comparisons between groups for categorical characteristics were carried out by the  $\chi^2$ -test (Yate's correction).

Spearman's correlation was used to assess the association of morning VEGF values with body mass index and polysomnography indices. To adjust body mass index for the effect of age and gender, relative body mass index [(absolute value/value of 50th percentile for age and gender)  $\times 100$ ] was calculated using standard growth curves.<sup>28</sup> To identify independent predictors of morning VEGF values, multiple linear regression analysis was performed (SPSS 10.0; SPSS, Chicago, IL, USA). Morning VEGF values were log-transformed prior to the analysis so that they were normally distributed. Log-transformed morning VEGF concentration was the dependent variable and AHI (or % sleep time with oxygen saturation of hemoglobin less than

90%), age, gender and relative body mass index were entered as independent variables.

## Results

### Patient characteristics and results of polysomnography

Eighty-five children with and 34 children without snoring were invited to participate in the study. Parents of 15 children (eight snorers and seven non-snorers) declined participation to the study and four more subjects (two snorers and two non-snorers) were excluded due to symptoms of upper respiratory tract infection. A total of 100 subjects were finally recruited in the study: 20 children with habitual snoring and  $\text{AHI} \geq 5$  episodes/h (median age 5; range 1.9–13 years); 55 subjects with snoring and  $\text{AHI} < 5$  episodes/h (median age 6; range 2–13 years); and 25 controls without snoring (median age 6.5; range 3–13 years). Frequency of sleep-disordered breathing symptoms in the three study groups, and polysomnography indices in children with an  $\text{AHI} \geq 5$  episodes/h or  $\text{AHI} < 5$  episodes/h are summarized in Table 1.

### VEGF measurements

The study groups were similar regarding variables that may affect VEGF values (Table 2). No significant differences were identified between groups regarding evening or morning VEGF values ( $P > 0.05$ ) (Table 2 and Fig. 1). Mean ( $\pm$ sd) morning VEGF values were:  $35.4 \pm 42.5$  pg/mL in children with snoring and  $\text{AHI} \geq 5$  episodes/h;  $46.7 \pm 54.5$  pg/mL in subjects with snoring and  $\text{AHI} < 5$  episodes/h; and  $50.7 \pm 37.1$  pg/mL in controls without snoring. The overnight change in VEGF levels in children with habitual snoring was also similar in: snorers with  $\text{AHI} \geq 5$  episodes/h (median 0 pg/mL; range  $-94.7$  to  $72.6$ ); snorers with  $\text{AHI} < 5$  episodes/h (median 0 pg/mL; range  $-108.6$  to  $138.2$ ); and controls without snoring (median 10.4 pg/mL; range  $-67.2$  to  $101.6$ ) ( $P > 0.05$ ).

Morning VEGF levels were not correlated with: (i) relative body mass index ( $r = -0.04$ ;  $P > 0.05$ ); (ii) AHI ( $r = -0.15$ ;  $P > 0.05$ ); (iii) respiratory arousal index ( $r = -0.17$ ;  $P > 0.05$ ); (iv) oxygen saturation of hemoglobin nadir ( $r = 0.04$ ;  $P > 0.05$ ); (v) oxygen desaturation of hemoglobin index ( $r = -0.2$ ;  $P > 0.05$ ); or (vi) % sleep time with oxygen saturation of hemoglobin less than 90% ( $r = -0.06$ ;  $P > 0.05$ ). When multiple linear regression analysis was carried out, AHI (or % sleep time

**Table 1** Summary statistics for symptoms of sleep-disordered breathing and polysomnography indices in the three study groups.

	Snorers with AHI $\geq 5$ episodes/h ( <i>n</i> = 20)	Snorers with AHI < 5 episodes/h ( <i>n</i> = 55)	Controls without snoring ( <i>n</i> = 25)
Snoring > 3 nights/week	20 (100%)	55 (100%)	0 (0%)
Apnea > 3 nights/week	16 (80%)	28 (51%)	0 (0%)
Difficulty breathing > 3 nights/week	17 (85%)	37 (67%)	0 (0%)
Apnea index (episodes/h)	2.9 (0.7–16.7)	0.6 (0–2.3)	—
Apnea–hypopnea index (episodes/h)	9.6 (5–45.4)	1.9 (0.2–4.9)	—
Respiratory movement/arousal index (episodes/h)	2.2 (0.7–16.3)	0.7 (0–2.2)	—
Oxygen desaturation of hemoglobin (> 4%) index (episodes/h)	9.4 (3.3–49)	1.5 (0–4.4)	—
SaO <sub>2</sub> nadir (%)	84 (69–91)	91 (81–94)	—
% Sleep time with SaO <sub>2</sub> < 90%	0.3 (0–5.6)	0 (0–0.26)	—

Continuous variables are expressed as median (range).

AHI: Apnea–hypopnea index.

**Table 2** Summary statistics and significance of comparisons between the three study groups regarding vascular endothelial growth factor (VEGF) values and factors that may affect these values.

	Snorers with AHI $\geq 5$ episodes/h ( <i>n</i> = 20)	Snorers with AHI < 5 episodes/h ( <i>n</i> = 55)	Controls without snoring ( <i>n</i> = 25)
Age (years)	5 (1.9–13)	6 (2–13)	6.5 (3–13)
Gender, female (%)	9 (45%)	26 (47.3%)	11 (44%)
Relative BMI (%)	102.5 (71.5–147.3)	115.5 (86.2–180.8)	105.9 (78.2–184.6)
Evening VEGF (pg/mL)	2.5 (2.5–174.5)	22.5 (2.5–159.4)	26.8 (2.5–108)
Morning VEGF (pg/mL)	7.7 (2.5–120.5)	25.1 (2.5–198.4)	48.4 (2.5–147.7)

Continuous variables are presented as median (range).

*P* > 0.05 for all comparisons between study groups.

BMI: Body mass index.

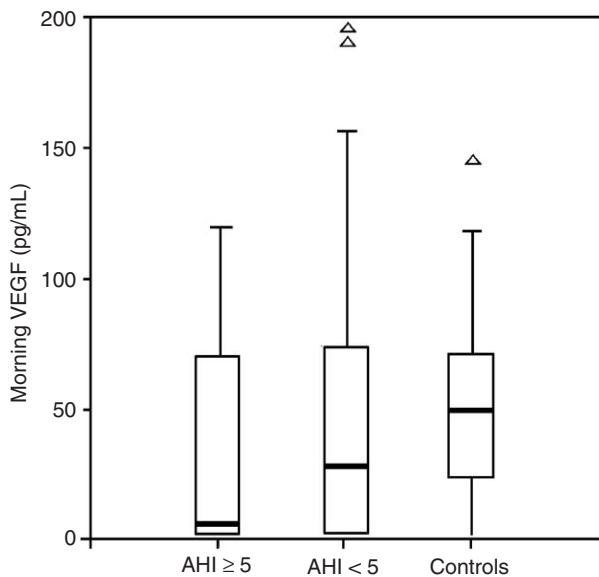
with oxygen saturation of hemoglobin less than 90%), age, gender and relative body mass index were not significant predictors of log-transformed morning VEGF values.

## Discussion

In the present investigation, evening and morning plasma VEGF levels were measured in children with obstructive sleep-disordered breathing of variable

severity and in controls without snoring. In accordance with findings reported in adults with obstructive sleep apnea by Valipour and colleagues,<sup>22</sup> similar levels of VEGF were identified in the three study groups that were not correlated with severity of sleep-disordered breathing. No differences were noted in the overnight change of VEGF values between the three groups of participants.

Obstructive sleep apnea–hypopnea in adults has been correlated with increased frequency of cardiovascular disease and various etiologic links have been proposed to explain this association.<sup>1–5</sup>



**Figure 1** Boxplots of morning plasma vascular endothelial growth factor (VEGF) levels (pg/mL) in: (i) children with habitual snoring and apnea-hypopnea index (AHI)  $\geq 5$  episodes/h ( $n = 20$ ); (ii) subjects with habitual snoring and AHI  $< 5$  episodes/h ( $n = 55$ ); and (iii) controls without snoring ( $n = 25$ ). VEGF values are similar in the three study groups ( $P > 0.05$ ). Horizontal bars represent median VEGF levels; upper and lower parts of the boxplots are the 25th and 75th percentile values; whiskers represent highest and lowest VEGF levels and triangles are outlier values.

Persistent long-term increase of VEGF concentrations may be one of the factors participating in the process of atherogenesis and development of cardiovascular disease in subjects with obstructive sleep apnea-hypopnea and nocturnal hypoxemia.<sup>12,13,16–20</sup> Nevertheless, increased VEGF levels are not identified in all adults with sleep apnea.<sup>22</sup>

Serum VEGF levels are similar in healthy adults and children.<sup>20,29</sup> VEGF is a key regulator in angiogenesis, and hypoxia is a major stimulus for its synthesis.<sup>30,31</sup> Administration of 2 L/min of oxygen overnight to adults with sleep apnea and hypoxemia was associated with a significant decrease in serum levels of VEGF.<sup>19</sup> Gozal and colleagues<sup>20</sup> reproduced findings of studies with adult participants in 20 children with obstructive sleep apnea (apnea index  $> 5$  episodes/h). Children with an apnea index  $> 5$  episodes/h had higher morning serum VEGF concentrations than controls with apnea index  $< 5$  episodes/h ( $n = 21$ ) ( $220 \pm 112$  versus  $66 \pm 23$  pg/mL). VEGF levels were correlated with both respiratory disturbance index and duration of nocturnal hypoxemia, which is in contrast to our findings (absence of correlation with polysomnography indices).

When comparing the present report with the investigation by Gozal and colleagues,<sup>20</sup> mean ( $\pm$ SD) morning VEGF concentrations in children with sleep-disordered breathing of the former study seem to be lower than VEGF levels in subjects of the latter study. These different values could be due at least partially to measuring plasma VEGF levels in the current study as opposed to measuring serum levels in the previous report.<sup>20,21</sup> More subjects were recruited in the present series than in the investigation by Gozal and colleagues (100 versus 41), whereas in both reports age and female-to-male ratio of participants were similar. Of note, children with snoring in the investigation by Gozal and colleagues had more severe and prolonged nocturnal hypoxemia. This study characteristic could possibly explain the difference in VEGF concentrations between subjects with obstructive sleep apnea and controls in the Gozal and colleagues report and the lack of such a difference between participants with AHI  $\geq 5$  episodes/h and controls in the current study.

No correlation was identified between morning VEGF levels and relative body mass index in this investigation and such a relationship was not described in the previously published pediatric report.<sup>20</sup> However, VEGF concentration was significantly related to body mass index in some,<sup>18,22</sup> but not all<sup>17</sup> reports including adults with sleep apnea.

A potential limitation of the current study is that controls without snoring did not undergo polysomnography because this was not acceptable to the majority of parents. We cannot exclude the possibility that more participants had to be included for identifying a significant relationship between nocturnal hypoxemia and morning VEGF levels, although we recruited far more children compared to the study by Gozal and colleagues.<sup>20</sup>

In conclusion, no significant association was identified between morning plasma VEGF levels and severity of obstructive sleep-disordered breathing in children with habitual snoring. In accordance with findings in adults,<sup>22</sup> VEGF may not play an important pathophysiologic role in all cases of obstructive sleep-disordered breathing in childhood.

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